PEPTIDES AND METHODS FOR DEACTIVATION OF ORGANOPHOSPHORUS-BASED NERVE AGENTS AND INSECTICIDES

This application claims priority of U.S. Serial No.
5 60/431,897, filed December 9, 2002, the content of which is
hereby incorporated into this application by reference.

The invention disclosed herein was made with United States
government support under grant numbers TLN 10-122 and DO
10 0788 from the Department of the Army, Army Research Office
1132; Scientific Services Program. Accordingly, the United
States government has certain rights in this invention.

Throughout this application, various publications are
15 referenced by author and date. Full citations for these
publications may be found at the end of the specification
immediately preceding the claims. The disclosures of these
references in their entireties are hereby incorporated by
reference into this application to describe more fully the
20 art to which this invention pertains.

Background of the Invention

Irreversible inhibitors of acetylcholinesterase (AChE) are
used extensively as insecticides and have also been used as
chemical weapons. World-wide stockpiles of these agents for
25 use in chemical warfare are estimated to exceed 200,000 tons
(Lejeune et al, 1998). The extreme toxicity of these
compounds and their global proliferation has made the
development of protective agents and antidotes an important
research priority.

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AChE inhibitors are derivatives of phosphoric,
pyrophosphoric and phosphonic acids. These agents react to

form a phosphoester with the serine residue that resides in the active site of the AChE enzyme. This phosphoester form of the enzyme is inactive, although the active enzyme can be regenerated through hydrolysis of the phosphoester.

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The toxicity of the organophosphorus agents arises from the stability of the phosphoester intermediate that is formed with the enzyme. As its name implies, AChE functions to cleave the ester linkage of acetylcholine, forming acetic
10 acid and choline. To do this, the enzyme transiently forms an ester with the carboxyl moiety of acetylcholine, releasing choline. The resulting carboxylate ester of the enzyme is inactive. However, this ester undergoes rapid hydrolysis, measured in milliseconds, to regenerate the
15 original, active form of the enzyme. In contrast, the rates of hydrolysis for the phosphate esters of AChE are measured in hours. The reversible inhibitors of AChE that are used clinically generate a carbamylated enzyme and have rates of hydrolysis measured in minutes. Thus, the time required for
20 regeneration of the active enzyme via hydrolysis of the ester linkage of the inactive, acetylated enzyme defines the difference between the enzyme's normal function, reversible inhibition, and irreversible inhibition.

25 One strategy for developing antidotes to irreversible AChE inhibitors has been the synthesis of highly nucleophilic small molecules capable of efficiently cleaving phosphate esters. Hydroxylamine is one such compound that was demonstrated to significantly increase the rate of
30 hydrolysis of phosphorylated AChE. However, efficient hydrolysis was only achieved at toxic concentrations of hydroxylamine.

To date, this approach has yielded only one compound that has shown clinical efficacy, 2-pyridine aldoxime methylchloride (2-PAM). The oxygen atom of this molecule is part of an oxime functional group. The oxime moiety is a hydroxylamine-like nucleophile formed from the reaction of hydroxylamine with aldehydes or ketones. The effectiveness of this compound is limited by its inability to cross the blood-brain barrier. However, effectiveness is further impaired by an inability to regenerate the enzyme once "aging" has occurred. This latter impediment means that treatment is only effective if administered within a few minutes to a few hours of toxin exposure, depending on the toxin.

Organophosphorus agents, particularly some of the more recent additions to chemical weapons arsenals, have the propensity to become truly irreversible inhibitors through the aging process. This molecular process occurs when, having first reacted with AChE to inactivate the enzyme, a phosphoester bond undergoes cleavage, resulting in an anionic ester which is extremely resistant to hydrolysis. The phosphorylated enzyme which has aged in this way is completely refractory to regeneration by currently available antidotal agents, including 2-PAM. It is this aging process that makes the phosphorus-derived chemical warfare agents, such as sarin, soman, VX and tabun, extremely lethal.

In addition to efforts to produce antidotes, research has focused on protecting against exposure to organophosphorus agents, thereby preventing or moderating their toxic effects. One important strategy has been the use of recombinant enzymes for the biocatalytic degradation of organophosphorus agents. This methodology is being utilized in the development of protective clothing as well as agents

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for surface or aerial decontamination. In these methodologies, microbial enzymes, such as cholinesterases or organophosphorus hydrolases, are attached to a solid support in a manner that retains some portion of their catalytic

5   activity. Such biocatalytic materials include silicone polymers (Gill 2000) and polyurethane foams (LeJeune 1996, 1999; Cheng 1996).

Peripheral blocking methods, i.e., methods that rely on

10   agents that would intercept nerve agents in the circulatory system before they partition into the central nervous system or muscle, are currently the only choice for protection against organophosphorus agents. The standard technology in use today for protection against nerve agents is based upon

15   the recombinant enzyme, butyrylcholinesterase (BuChE). BuChE rapidly reacts with nerve agents to form an intermediate phosphoester with a serine in the active site much as occurs when these agents react with AChE. Compounding the slow rate of hydrolysis of this intermediate

20   is the inactivation of the enzyme through the process of molecular aging discussed above. Thus, BuChE is essentially an autocatalytic stoichoeometric blocker rather than a true hydrolytic enzyme. Butyrylcholinesterase mutants demonstrate enzymatic turnover, but low turnover, combined with high

25   immunogenicity, an inability to cross the blood-brain barrier and a high equivalent weight ratio (almost 500:1 for butyrylcholinesterase to nerve gas). These factors make it difficult to maintain *in vivo* enzyme levels sufficient to protect against a lethal dose of a toxin such as a nerve

30   agent.

To date, the above-mentioned shortcomings have not been overcome.

Summary of the Invention

This invention provides a method for determining whether a peptide forms a phosphorus-based ester with an organophosphorus agent. This invention also provides a

5   method for determining whether, among a plurality of peptides, there exists a peptide that forms a phosphorus-based ester with an organophosphorus agent. This invention further provides a method for identifying and characterizing a peptide, among a plurality of peptides, that forms a

10. phosphorus-based ester with an organophosphorus agent. This invention further provides a peptide which forms a phosphorus-based ester with an organophosphorus agent, which peptide comprises a nucleophilic functional group. This invention also provides a peptide library, wherein each

15   peptide therein comprises a nucleophilic functional group. This invention further provides a composition of matter comprising a peptide and a pharmaceutical or a nonpharmaceutical carrier. This invention also provides an article of manufacture comprising a peptide affixed to a

20   solid substrate.

This invention further provides a method for reducing the likelihood of injury due to exposure to an organophosphorus-containing agent in a subject exposed to or at risk of

25   exposure to such agent, comprising administering to the subject an effective amount of a peptide of the instant invention.

This invention also provides a method for decontaminating an

30   area exposed to an organophosphorus-containing agent comprising introducing to the area an effective amount of a peptide of the instant invention. This invention further provides a kit for decontaminating an area exposed to an

organophosphorus-containing agent comprising the instant peptides and instructions for use.

Finally, this invention provides a method for determining the presence of an organophosphorus-containing agent in an area.

Brief Description of the Figures

Figure 1: A sarin analog tethered to a fluorescent label was synthesized as shown to provide a low toxicity version of soman that would retain its chemical reactivity and report its reaction with a peptide molecule displayed on a library bead.

Figure 2: Structure of the fluorescently labeled sarin analog (1) and additional analogs with the following properties, hydrophobicity (2), water-solubility (3) and UV-activity (4).

Figure 3A: Analog (2) was synthesized by fluoration of 1-(isopropylhydroxyphosphinyl)-10-undecene with DAST.

Figure 3B: Synthesis of analog (3). The olefin (5) was oxidatively converted to aldehyde (6) with OsO_4 and $NaIO_4$. Reductive amination of (6) with dimethyl amine and sodium borohydride afford the amine (7). Quaternazation of the tertiary amine (7) with iodomethane in DMF gave (8) which was treated with DAST to afford the analog (3).

Figure 3C: Synthesis of analog (4). Treatment of phenylphosphonic dichloride with excess isopropanol in the presence of triethylamine afforded the diisopropyl phenylphosphonate which was subjected to basic hydrolysis with NaOH to generate the monoester (isopropyl phenylphosphonic acid). The nerve gas analog (4) was generated by treatment with DAST in a manner similar to analog (1).

Detailed Description of the Invention

Definitions

In this invention, "administering" can be effected or
performed using any of the various methods and delivery
5   systems known to those skilled in the art. The administering
can be performed, for example, intravenously, orally, via
implant, transmucosally, transdermally, intramuscularly, and
subcutaneously. In a preferred embodiment, the peptide is
administered intramuscularly.
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Determining an "effective amount" of the instant peptide for
decontamination purposes can be done based on in vitro data.
Determining an effective amount of the instant peptide for
administering to a subject can be done based on animal data
15   using routine computational methods. In one embodiment, the
effective amount contains between about 10 mg and 1000 mg of
the instant peptide. In another embodiment, the effective
amount contains between about 50 mg and about 500 mg of the
peptide. In a further embodiment, the effective amount
20   contains between about 100 mg and about 250 mg of the
peptide, and preferably about 200 mg thereof.

"Pharmaceutical carriers" are well known in the art and
include, but are not limited to, 0.01-0.1 molar phosphate
25   buffer, 0.8% saline solution, propylene glycol, polyethylene
glycol, vegetable oils and injectable organic esters such as
ethyl   oleate.   Aqueous   carriers   include   water,
alcoholic/aqueous   solutions,   emulsions   or   suspensions,
including saline and buffered media. Parenteral vehicles
30   include   sodium   chloride   solution,   Ringer's   dextrose,
dextrose and sodium chloride, lactated Ringer's or fixed
oils. Intravenous vehicles include fluid and nutrient

replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases

5 and the like.

"Nonpharmaceutical carriers" include, without limitation, foams and aerosols. For example, a polyurethane foam can be synthesized to incorporate a peptide of the instant

10 invention using methods standard in the art of polymer synthesis. In one example, a hydrophilic polyurethane prepolymer is reacted with water containing the peptide and a surfactant, forming a foam. Such prepolymers are known in the art and comprise a urethane capped with multiple

15 isocyanate functionalities. As one of skill in the art would be aware, various surfactants can be used in order to alter the porosity, density and surface properties of the foam.

The term "organophosphorus agent" is synonymous with

20 "organophosphorus-based agent", and is used herein to refer to a chemical compound comprising carbon, hydrogen, phosphorous and oxygen. Organophosphorus agents include, without limitation, phosphorus-based esters. A number of commercially important insecticides, as well as chemical

25 warfare agents, contain a phosphorus-based ester moiety which either is inherently toxic to humans or is readily converted into a toxic moiety. Examples of such agents include, without limitation, malathion, parathion, paraoxon, schradan, dichlorfenthion, soman, sarin, VX, GB and tabun.

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The term "phophorus-based ester" is used herein to refer to any ester of phosphorous. Examples of such esters include, without limitation, a phosphate ester, a phosphonate ester and a phosphinate ester.

The term "peptide" is used herein to refer to a polymer of amino acid residues. In one embodiment, a peptide has 15 or fewer amino acid residues. Amino acid residues include,
5   without limitation, any of the 20 naturally occurring amino acids or modifications thereof, as well as natural or synthetic derivatives thereof. A peptide can also comprise one or more modified amino acid residues. Modifications include, without limitation, the conjugation to an amino
10   acid residue of a non-protein moiety (e.g. an oxime or a cyclodextran). Other modifications include the addition of a chemical tag useful for identification or purification. Examples of chemical tags include, without limitation, a biotin molecule, a radioisotope and a fluorescent molecule.

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As used herein, a "solid substrate" and a "solid support" are synonymous, and shall mean any water- and/or lipid-insoluble support to which a peptide can be affixed. Examples of solid supports include, without limitation,
20   alumina pellets, trityl agarose and glass/silica beads, and polymers such as nylons, acrylates, and silicas.

"Subject" shall mean any animal, such as a primate, mouse, rat, guinea pig or rabbit. In the preferred embodiment, the
25   subject is a human

Embodiments of the Invention

This invention provides a method for determining whether a peptide forms a phosphorus-based ester with an organophosphorus agent comprising the steps of contacting
30   the peptide with the agent under conditions permitting the formation of a phosphorus-based ester and determining whether a phosphorus-based ester has formed.

This invention also provides a method for determining whether, among a plurality of peptides, there exists a peptide that forms a phosphorus-based ester with an organophosphorus agent. This method comprises the steps of
5   contacting the plurality of peptides with the agent under conditions permitting formation of a phosphorus-based ester and determining whether a phosphorus-based ester has formed.

This invention further provides a method for identifying and
10   characterizing a peptide among a plurality of peptides that forms a phosphorus-based ester with an organophosphorus agent. This method comprises the steps of contacting the agent with the plurality of peptides under conditions permitting the formation of a phosphorus-based ester,
15   identifying the peptide or peptides that form such an ester with the agent and determining the amino acid sequence of the peptide so identified.

In an embodiment of any of these methods, the
20   organophosphorus agent which reacts with a peptide is selected from the group consisting of malathion, parathion, paraoxon, schradan, dicholorfenthion, soman, sarin, VX, GB and tabun, or an analog thereof.

25   In any of these three methods, the phosphorus-based ester can be any ester of phosphorus including, for example, a phosphate ester, a phosphonate ester or a phosphinate ester.

In one embodiment of the instant methods, the peptides are
30   bound to a solid support such as a bead, a microtiterplate, a glass chip or a silicone chip. In another embodiment, the agent is labeled with a detectable marker. In a preferred embodiment, the detectable marker is a radioisotope, a fluorescent molecule, biotin or an enzyme. In one example,

the agent is a nerve agent and the detectable marker is
rhodamine. In another example, the agent has the structure
set forth as analog (1) in Figure 2, which is a rhodamine-
conjugated analog of the nerve agent soman.

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In another embodiment of the instant methods, the peptides
are not bound to a solid support. Instead, the agent or
peptide is labeled with a moiety that provides a basis for
affinity purification. Such moieties are well-known in the
10 art and include, for example, biotin and glutathione. Such
purification can comprise a step of removing unreacted
peptide and agent prior to affinity purification. This step
may be accomplished in a number of ways using techniques
that are well-known in the art, such as chemical separation
15 based on solubility, size exclusion chromatography and gel
electrophoresis.

This invention also provides a peptide which forms a
phosphorus-based ester with an organophosphorus agent, which
20 peptide comprises a nucleophilic functional group. In one
embodiment of this invention, the nucleophilic functional
group comprises a thiol or a hydroxyl group. In a preferred
embodiment, the peptide is between six and 15 amino acids in
length or has a molecular weight of less than 1500 daltons.
25 In one embodiment, the peptide is six amino acids in length.

In the preferred embodiment of this invention, the agent
with which the peptide reacts is an organophosphorus
insecticide or chemical warfare agent.

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This invention also provides a peptide library, wherein each
peptide therein comprises a nucleophilic functional group.
In one embodiment, each peptide in the library comprises a
thiol or hydroxyl-containing amino acid residue, and the

position at which such thiol or hydroxyl-containing amino acid residue occurs is the same for each peptide in the library. In another embodiment, each peptide is of a fixed length. In a further embodiment, the length of each peptide
5 is between six and 15 amino acid residues and/or the molecular weight of each peptide is less than 1500 daltons. In a further embodiment, each peptide has a length of six amino acid residues. In still another embodiment, the first, second, third, fourth, fifth, or sixth amino acid residue in
10 each hexapeptide is a serine.

This invention also provides a composition of matter comprising one of the instant peptides and a pharmaceutical carrier. This invention further provides a composition of
15 matter comprising one of the instant peptides and a nonpharmaceutical carrier. In one embodiment, the nonpharmaceutical carrier is a foam or aerosol.

Foam formulations and mechanisms for their dispersal are
20 well-known in the art and are particularly useful for surface decontamination. Foam formulations specifically designed for blast suppression are also known in the art and would be particularly useful in combination with the peptides of the instant invention in containing an explosive
25 device that has been designed to disseminate a nerve agent.

This invention also provides an article of manufacture comprising one of the instant peptides affixed to a solid substrate. In one embodiment, the solid substrate is a
30 polymer. In another embodiment, the solid substrate is a fabric or fiber. In yet another embodiment, the solid substrate is a filtration component, such as one used in a gas mask.

In the preferred embodiment, the peptides of the instant invention are incorporated into polymers using art-recognized techniques. Many different types of polymers are known in the art. The choice of polymer would depend, for
5   example, upon whether the peptide is to be incorporated into protective clothing, a device such as a gas mask or a pellet or sheet for surface decontamination.

This invention provides a method for reducing the likelihood
10 of injury due to exposure to an organophosphorus agent in a subject exposed to or at risk of exposure to such an agent. This method comprises administering to the subject an effective amount of a peptide of the instant invention.

15 This invention also provides a method for decontaminating an area exposed to an organophosphorus agent comprising introducing to the area an effective amount of a peptide of the instant invention.

20 This invention further provides a kit for decontaminating an area exposed to an organophosphorus agent comprising a peptide of the instant invention and instructions for use.

Finally, this invention provides a method for determining
25 the presence of an organophosphorus agent in an area. This method comprises the steps of contacting a peptide of the instant invention with a sample taken from the area and determining whether a phosphorus-based ester is formed with the peptide. In this method, the formation of such an ester
30 is indicative of the presence of an organophosphorus agent in the area.

This invention is illustrated in the Experimental Details section which follows. This section is set forth to aid in

an understanding of the invention but is not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

Experimental Details

Synopsis

The present invention provides methods and reagents for protection against poisoning by organophosphorus-based
5 insecticides and nerve agents. In a novel approach to producing such reagents, this invention utilizes combinatorial chemistry and high-throughput screening to generate catalytic peptides for the inactivation of organophosphorus-based toxins. The peptides provided by the
10 instant invention are selected based on their ability to autocatalytically bind and thereby inactivate substrate phosphoesters and offer several advantages over current enzyme-based approaches. These include decreased immunogenicity, and more favorable equivalent weight ratios.

15 ### Methods

Design of the peptide library

A combinatorial approach based on "split and pool" synthesis was used to generate a diverse population of peptides. Hexapeptide libraries were designed with five random
20 positions and one fixed serine, giving 3.2 million unique peptides per library. Separate libraries were generated that contained a serine at each position, $[x_1, x_2...x_6]$ in the peptide. Thus, a total of six libraries is produced, each having serine fixed in a different position of the
25 hexapeptide.

Synthesis of the peptide libraries

Tental Gel™ was used as the solid phase support and sequences of active peptides were determined using mass spectrometry (MS). Peptide library synthesis was
30 accomplished by a "split and pool" methodology using

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standard Fmoc peptide chemistry. For example, the first library, in which serine occupies the first position, x_1, was synthesized as follows: (i) the beads were reacted with Fmoc-protected serine using benzotriazol-1-

5   yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and hydroxybenzotriazole (HoBt) as coupling agents, (ii) amino groups were blocked with acetic anhydride, and (iii) the Fmoc protecting group was removed using 20% piperidine in DMF. The next five amino acids in the peptide

10   were placed randomly using the "split and pool" method. This is accomplished by separating the entire population of beads into twenty groups of equal size. Each of the twenty amino acids is reacted with an individual group using BOP and HoBt as coupling agents. After the coupling reaction, the beads

15   are combined so that they can be washed, blocked, and finally deprotected together. This split and pool synthesis is performed five times in order to add five random amino acids to each peptide.

Library screening

20   Libraries were exposed to a fluorescently labeled nerve gas analog for 1 hr followed by 1 minute washes at room temperature in PBS containing 10% Tween 20, 10% DMF, followed by DMF containing 10% Tween 20. Butylcholinesterase coupled to native beads was used as a positive control.

25   Library peptides which remained fully protected and thus unreactive were used as a negative control. Wash conditions were optimized to exclude noncovalent interactions between the fluorescent label and the beads utilizing either peptides in which the reactive groups were blocked by pre-

30   incubation with either acetic anhydride or a single "non-specific" peptide not likely not to be configured for reaction.

Following incubation with analog (1) and extensive washing, beads displaying relatively high levels of fluorescence were selected and their peptides sequenced using LCMS. Peptides corresponding to those identified in the screen were

5   synthesized using Tental Gel™ as the solid phase and standard Fmoc chemistry, then purified using high-pressure liquid chromatography (HPLC).

Synthesis of fluorescent sarin analogs

Fluorescent-labeled sarin analogs were synthesized as shown

10 in Fig. 1. Briefly, 10-undecen-1-ol (1) was converted to an iodinated compound (3) through a tosylate intermediate (2). Reaction of (3) with excess tri-isopropylphosphite under reflux conditions afforded the di-isopropoxy phosphonate (4), which was converted to the isopropoxyhydroxy

15 phosophonate (5) by refluxing with NaOH in methanol. The olefin (5) was oxidatively cleaved with ruthenium trichloride and sodium periodate to yield the terminal carboxylic acid (6). Treatment of (6) with excess diethylaminosulfur trifluoride (DAST), followed by N-

20 hydroxysuccinimide (NHS), afforded the N-succinyl fluorophosphonate intermediate (8). Without further purification compound (8) was reacted with amine-functionalized rhodamine (NH_2-rhodamine) to generate the rhodamine-labeled sarin analog (9). The structures of the

25 compounds 2-8 and 9 (analog 1) were determined by NMR and MS. Experimental details are set forth below.

Compound (2) [1-((*p*-toluenesulfonyl)oxy)-10-undecene]:   A solution of (1) (5 g, 0.029 mol) in pyridine (50 ml) was

30 reacted with *p*-toluenesulfonyl chloride (8.2 g, 0.044 mol) at room temperature for 4 h. The reaction mixture was evaporated and the residue was dissolved in ethyl acetate. The acetate solution was washed with 1N HCl (2x30ml) and

concentrated under reduced pressure. The product (2) was purified by chromatography (SiO$_2$, ethyl acetate/hexane 1/20). Colorless oil, yield 8.9 g, 94%, ^{1}H NMR (CDCl$_3$, 300MHz): 7.7(d, 2H), 7.32(d, 2H), 5.8(m, 1H), 5.0(m, 2H), 4.0(t, 2H), 5 2.4(s, 3H), 2.0(m, 2H), 1.7(m, 2H), 1.5-1.2(m, 12H); MS: found 324.18, cald. 324.18 for C$_{18}$H$_{28}$O$_3$S.

Compound (3) [1-iodo-10-undecene]: A solution of (2) (8.0 g, 0.025 mol) in acetone (80 ml) was treated with NaI (7.0 g, 10 0.05 mol). The reaction mixture was refluxed for 2 h and concentrated. The residue was dissolved in ethyl acetate (50 ml) and washed with H$_2$O (20 ml). The product (3) was purified by chromatography (SiO$_2$, ethyl acetate/hexane 1/20). 5.5 g oil, 79%. ^{1}H NMR (CDCl$_3$, 300 MHz): 5.8(m, 1H), 5.0(m, 2H), 15 3.2(t, 2H), 2.0(m, 2H), 1.8 (m, 2H), 1.50-1.20(m,12H). MS: found 280.07, cald. 280.07 for C$_{11}$H$_{21}$I.

Compound (4) [1-(di-isopropoxy) phosphinyl)-10-undecene]: A mixture of triisopropyl phosphite (10 ml, excess) and 20 compound (3) (5.0 g) was refluxed for 15 h. Excess of triisopropyl phosphite was removed by distillation under reduced pressure. The product was purified by chromatography (SiO$_2$, ethyl acetate/hexane ¼). Oil, 5.4 g, 93%. ^{1}H NMR (CDCl$_3$, 300 MHz) 5.70 (m, 1H), 4.9 (m, 2H), 4.80 25 (m, 2H, CHOP), 2.0-1.0 (m, 30H). MS: found 318.380, cald. 318.380 for C$_{17}$H$_{35}$O$_3$P.

Compound (5) [1-(isopropylhydroxyphosphinyl)-10-undecene]: A solution of compound (4) (5.0 g, 0.016 mol) in methanol (80 30 ml) and aqueous NaOH (5.0 g in 15 ml H$_2$O) was refluxed for 5 h. The solvents were removed by concentration under reduced pressure. The residue was dissolved in H$_2$O (30 ml) and extracted with ethyl ether. The aqueous solution was acidified with 6 N HCl to pH=2, and extracted with ethyl

acetate. The organic phase was evaporated to give pure compound (5) as clear oil. 3.9g, 89%. ^{1}H NMR (CDCl$_3$, 300 MHz) 5.70 (m, 1H), 4.9 (m, 2H), 4.80(m, 1H, CHOP), 2.0-1.0 (m, 24H).

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Compound (6) [10-(iso-propylhydroxyphosphinyl) decanoic acid]: To compound (5) (1.0g, 3.6 mmol) dissolved in CCl$_4$-CH$_3$CN-H$_2$O (1:1:1.5)(35 ml) was added NaIO$_4$ (3.1g, 4 eq.) and ruthenium trichloride hydrate (20 mg). The reaction mixture was stirred for 2 h at room temperature and then partitioned between CH$_2$Cl$_2$ (40 ml) and 1N HCl (20 ml). The organic layer was concentrated under reduced pressure and the resulting residue was purified by chromatography to give semisolid product (SiO$_2$, ethyl acetate/methanol ½). 0.7 g, 70%. ^{1}H NMR (CDCl$_3$) 4.70 (m, 1H, CHOP), 2.3 (t. 2H), 2.0-1.0 (m, 24H). High resolution MS: found 295.166, cald. 295.166 for C$_{13}$H$_{28}$O$_5$P.

Compound (9) [Rhodamine-labeled sarin analog (1)]: A solution of (6) (100 mg, 0.34 mmol), in CH$_2$Cl$_2$ (3 ml) was treated at -78°C with diethylaminosulfur trifluoride (DAST, 0.1 mL, 0.5 mmol), and the reaction mixture was stirred for 10 min. The resulting difluoride (7) was treated in situ with N-hydroxysuucinimide (0.4 mmol) in DMF (0.2 ml) and stirred for another 5 minutes at room temperature. The reaction mixture was partitioned between ethyl acetate (15 ml) and water (10 ml), and the organic layer was washed with brine (5 ml), dried with Na$_2$SO$_4$ and concentrated under reduced pressure to afford the N-hydroxysuccinimide (8). Without further purification, this compound was treated with amine-functionalized rhodamine derivative (1.0 eq) in DMF (2 ml) and stirred for 20 minutes. The solvents were evaporated under reduced pressure. The remaining residue was washed sequentially with diethyl ether and ethyl acetate to afford

(9) as a red film. 150 mg, 49%, ^{1}H NMR (CDCl$_3$, 300 MHz) 7.0-8.0 (m, 9H), 4.90 (m, 1H, CHOP), 2.5-1.0 (m, 46H). High resolution MS: found 793.4105, cald. 793.4400 for C$_{43}$FH$_{59}$N$_4$O$_7$P.

5   *Synthesis of additional sarin analogs*

Synthesis of analog (2): Analog (2) was synthesized by fluoration of compound (5) (Figure 1), 1-(isopropylhydroxyphosphinyl)-10-undecene, with DAST in a similar manner to that of analog (1) shown in Figure 1 and

10   described above. The synthetic scheme of analog (2) is summarized in Figure 3A.

Synthesis of analog (3): Analog (3) was synthesized by the route outlined in Scheme 2, Figure 3B. The olefin (5) was

15   oxidatively converted to an aldehyde (6) with OsO$_4$ and NaIO$_4$. Reductive amination of (6) with dimethyl amine and sodium borohydride afforded the amine (7). Quaternazation of the tertiary amine (7) with iodomethane in DMF gave (8) which was treated with DAST to afford the analog (3).

20

Experimental details for analog (3): Compound (5) (0.74 g) in a solution of THF (10 ml) H$_2$O (6 ml) was added to 2% OsO$_4$ in H$_2$O (2 ml) and NaIO$_4$ (2.0 g, 4 eq.). The mixture was stirred at room temperature for 18 h, quenched with

25   saturated Na$_2$SO$_3$ (10 ml) and partitioned with ethyl acetate. Evaporation of the solvents afforded the aldehyde (6) [oil, 0.5 g. 70% by NMR]. The aldehyde (0.45 g) (6) in ethanol (50 ml) was added to dimethylamine (1.3 eq). To this solution was added NaBH$_4$ (1.1 eq). The reaction mixture was

30   stirred for 4 h and the treated with 1 N HCl at 0°C. After removal of the solvents, the residue was dissolved in water and washed with ethyl ether. The aqueous layer was concentrated under reduced pressure to afford compound (7)

[0.32 g, 63% by NMR]. The amine (7) (0.3 g) in DMF (10 ml) was treated with MeI (1 ml, excess) overnight. The solvent was removed under reduced pressure to give compound (8) which was converted to analog (3) in a similar manner as
5   analog (1) [semisolid, 85% by NMR].

Synthesis of analog (4): Analog (4) was synthesized by the route outlined in scheme 3, Figure 3C. Treatment of phenylphosphonic dichloride with excess isopropanol in the
10 presence of triethylamine afforded the diisopropyl phenylphosphonate which was subjected to basic hydrolysis with NaOH to generate the monoester (isopropyl Phenylphosphonic acid). The nerve gas analog (4) was generated by treatment with DAST in a manner similar to
15 analog (1).

Solution-phase reaction of peptides with sarin analogs

1 mmol of purified peptide was incubated with one of the nerve agent analogs (1.2 mmol) in PBS at room temperature for 2-3 hr. The formation of reaction product was monitored
20 by HPLC (C_{18} analytic column, gradient eluting with 100% H_2O--70% MeCN for 1 h) and confirmed by mass spectrophotometry.

Results and Discussion

In order to obtain catalytic peptides with the desired reactivity, libraries consisting of peptides containing a
25 nucleophilic moiety were conceived which would react with the phosphonate moiety of organophosphorus-based agents to form a peptide phosphoester.

Initial work was done using hexapeptide libraries consisting
30 of a serine at a fixed position [$x_1, x_2, \ldots x_6$] and any amino acid at each of the remaining five positions. In one example, the hydroxyl moiety of serine serves as the

nucleophile which reacts with the phosphonate of the agent
to form a peptide-phosphoester, effectively blocking the
ability of the agent to react with cellular proteins and
thus neutralizing it.

5

Each of the six hexapeptide libraries consists of at least
3.2 million peptides comprised of a random amino acid at
each of five positions and a serine at a fixed position
(given by probability $20^5 = 3.2 \times 10^6$). For example, Library
10 1 consists of at least 3.2 million peptides, each having a
serine in position one (x_1) and any amino acid in each of the
remaining five positions. In practice, each peptide was
represented by approximately 6 copies in the library.
Initially, three libraries were synthesized containing a
15 fixed serine at either x_1, x_2, or x_3, respectively.

In order to identify peptides with the desired reactivity,
labeled analogs of the nerve gas sarin were synthesized.
Labeled butylcholinesterase (BuChE) coupled to the same
20 solid support as the library (in this case Tental Gel™) was
used as a positive control to identify peptides with
suitable reaction kinetics.

Although radiolabeled analogs are preferable because they
25 minimize interference due to steric hinderance, such analogs
of nerve agents present unacceptable hazards. Therefore,
fluorescent sarin analogs were generated using each of
rhodamine, fluorescein, cascade blue, dansyl cadaerine and
disperse red as labels. Preliminary experiments using native
30 beads as negative controls demonstrated that rhodamine-
conjugated sarin analog (1) produced the optimal signal to
noise ratio.

Library screening was performed with analog (1) and fluorescent beads were selected. Initially, beads demonstrating the highest fluorescence intensity were manually selected under the microscope. However, this
5 technique could easily be optimized for selection using high-throughput methods such as flow cytometry (for libraries coupled to beads), plate readers (for libraries adsorbed onto microtiter plates) or microarray scanners (for libraries on microchips).
10
23 of the manually selected fluorescent beads were chosen for sequencing and a further subset of ten of the resulting peptides was chosen for resynthesis and further study. These ten peptides had the following sequence: YKDNSY (SEQ ID
15 NO:1), YKDISY (SEQ ID NO:2), DNFKSY (SEQ ID NO:3), ANKYSY (SEQ ID NO:4), YYCDSY (SEQ ID NO:5), YHYYSY (SEQ ID NO:6), ANYYSY (SEQ ID NO:7), YEYQSY (SEQ ID NO:8), LIFASY (SEQ ID NO:9), YKEFSY (SEQ ID NO:10), and CAYCSY (SEQ ID NO:11).

20 Purified peptides identified in the initial screen were further tested for reactivity against sarin analog (2), which lacked the fluorescent tag. Using HPLC to monitor the reaction, one peptide was found to react with analog (2), demonstrated by the loss of its characteristic peak and the
25 emergence of three new chromatographic peaks. YKDNSY disappeared at retention time 17 min, and the 3 new peaks appeared at 23, 25 and 29 min. ^{1}H NMR analysis demonstrated that the peaks at 4.9 ppm (H for $Me_2CHP(O)F$) shifted to 4.5 ppm (H for $Me_2CHP(O)O$), indicating formation of a
30 phosphoester with the sarin analog. Diastereomers likely account for two of the products.

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